

PRONUCLEAR TRANSFER IN MICE YIELDS MINIMAL MITOCHONDRIAL DNA CARRY-OVER

Mado Vandewoestyne¹, Jitesh Neupane², Björn Heindryckx², Sylvie Lierman², Dieter Deforce¹, Petra De Sutter²

¹Laboratory of Pharmaceutical Biotechnology, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, 9000 Ghent, Belgium

²Department for Reproductive Medicine, Ghent University Hospital, De Pintelaan 185, 9000 Ghent, Belgium

Objective

Mitochondrial DNA (mtDNA) mutations can cause a wide range of severe diseases and are maternally inherited. Due to the lack of curative therapy, different strategies to prevent transmission of the mutation carrying mtDNA are being analyzed. One of these is the application of pronuclear transfer (PNT) to healthy cytoplasm.

Design

In this study, the potential of PNT was assessed in mice by measuring the mtDNA carry-over in pre-implantation embryos.

Materials and Methods

Pronuclei from zygotes of B6D2/F1 mouse were transplanted into enucleated NZB/OlaHsd mouse zygotes and vice versa to determine the quantity of mtDNA carried over in reconstructed zygotes as well as in individual blastomeres.

The mtDNA carry-over was determined by restriction fragment length polymorphism (RFLP) analysis.

Results

Mean percentage of mtDNA carry-over in reconstructed zygotes and blastomeres was 1.26 (± 1.83)% and 0.94 (± 1.39)%, respectively. The carry-over range was similar in the reconstructed zygotes and blastomeres with a minimal carry-over of 0% and a maximal carry-over of 4.47%. In 10 out of 17 samples, no mtDNA carry-over was detected (Table 1).

Celltype after PNT	N	mtDNA carry-over (%)	Mean (\pm SD) (%)
Reconstructed zygotes (n = 7)	1	3.09	1.26 (\pm 1.83)
	2	0	
	3	0	
	4	0	
	5	1.28	
	6	0	
	7	4.47	
2-cell embryos (n = 5 times 2)	1	0	0
	2	0	0.76 (\pm 1.08)
	1	1.52	
	2	0	0.86 (\pm 1.22)
	1	0	
	2	1.73	2.04 (\pm 2.89)
	1	4.08	
	2	0	1.04 (\pm 1.47)
	1	0	
	2	2.08	

Table 1: mtDNA carry-over (in %) in reconstructed zygotes and blastomeres after PNT in mice (Zygotes 1-6: PNT from B6D2 to NZB mouse; zygote 7: PNT from NZB to B6D2 mouse; all blastomeres from 2-cell embryos are PNT from B6D2 to NZB mouse)

Conclusions

Our data show that there is almost no or minimal mtDNA carry-over after PNT. This technique could potentially be used to prevent transmission of mtDNA mutation disorders from mothers to their offspring, providing hope for diseased parents to fulfil their desire of conceiving healthy babies.

Support

This Research was funded by a PhD grant and a postdoctoral grant from the Special Research Foundation (BOF) of Ghent University, Belgium, awarded to J.N. and M.V., respectively. P.D.S. is the holder of a fundamental clinical research mandate by the Flemish foundation of scientific research (FWO-Vlaanderen).